

SHOOT REGENERATION FROM HORMONE-AUTONOMOUS CALLUS FROM SHOOT CULTURES OF SEVERAL SUGARBEET (*BETA VULGARIS* L.) GENOTYPES*

J.W. SAUNDERS^a and M.E. DAUB^{b,**}

^aResearch Geneticist, USDA-ARS and ^bResearch Associate, Department of Crop and Soil Sciences, Michigan State University, East Lansing, MI 48824-1114 (U.S.A.)

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SUMMARY

Shoot cultures of six sugarbeet genotypes produced callus when grown in dim fluorescent light ($10\text{--}15\ \mu\text{Em}^{-2}\ \text{s}^{-1}$) at 30°C on Murashige-Skoog (MS) medium with $0.25\ \text{mg/l}$ 6-benzyladenine (BA). Hormone autonomy of the callus was indicated by sustained callus growth following multiple transfers onto MS medium lacking auxin and cytokinin. Shoots were regenerated from 1-month-old callus of two genotypes after transfer to MS medium with $0.25\text{--}5.0\ \text{mg/l}$ BA at 30°C in dim fluorescent light ($10\text{--}15\ \mu\text{Em}^{-2}\ \text{s}^{-1}$).

Key words: Hormone-autonomy — *Beta vulgaris* — Callus — Shoot regeneration — Shoot cultures

INTRODUCTION

The most noteworthy items about adventitious shoots on sugarbeet tissues to date have been the broad array of source tissues and the circumstances surrounding their appearance. Adventitious shoots in sugarbeet in vitro have been observed at the base of flower buds [1], at the base of isolated stem axes [2], on leaf pieces from shoots grown in vitro [3], and on intact leaves of shoot cultures [4,5]. In addition, regeneration of shoots in vitro has been observed from callus derived from anthers [6], seedling

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**Present address: Department of Plant Pathology, North Carolina State University, Raleigh, NC 27650, U.S.A.

Abbreviations: BA, 6-benzyladenine; IAA, indole-3-acetic acid; MS, Murashige and Skoog; NAA, α -naphthaleneacetic acid.

explants [7,8], axillary buds in vitro [9], as well as in an habituated cell line [10]. Adventitious shoots in sugarbeet have also been reported to occur at low frequency on leaf cuttings [11], and can also be induced on subsequent leaves of intact plants after benzyladenine treatment of seedlings [12]. We now report a reproducible method for induction of hormone-autonomous callus in sugarbeet and regeneration of shoots from this callus.

MATERIALS AND METHODS

Shoot cultures of seven genotypes were established from seedlings or lateral buds of flower stalks [4,5]. Murashige and Skoog [13] medium (MS) with 0.25 mg/l BA, 1.0 mg/l thiamine-HCl, 0.5 mg/l pyridoxine-HCl, 0.5 mg/l nicotinic acid and 0.9% (w/w) agar was used for routine maintenance and experimental culture. The medium was contained in Falcon Optilux 100 × 20 mm petri dishes with 35–40 ml per plate. Shoots were subcultured at monthly intervals, with three shoots transferred to each dish. Shoot cultures were maintained at $24 \pm 2^\circ\text{C}$ in growth chambers with continuous fluorescent (General Electric 40D Mainlighter) light at $100\text{--}200 \mu\text{Em}^{-2} \text{ s}^{-1}$.

Callus was cultured in petri dishes or in 25 × 70 mm glass screw-top vials (seals removed) containing 10 ml of medium. All callus cultures were grown on MS medium with differing concentrations of growth regulators, in lighted growth chambers (20° or 30°C) or in dark incubators (24°C). Callus growth was measured by the following procedure: 8-mm³ callus pieces were subcultured onto fresh medium. After 1 month, the length and width of the callus pieces were measured, and an approximate cross-sectional area was computed. Alternatively, callus fresh weight was recorded at subculture and again after 1 month. Shoots obtained in regeneration studies were subcultured on MS + 0.25 mg/l BA for 1 month, rooted on MS + 3.0 mg/l α -naphthalene-acetic acid (NAA) for 5 weeks, then potted in soil in the greenhouse.

RESULTS

Callus formation from shoot cultures

Sugarbeet shoot cultures that had been established for more than a year developed a white friable callus after 6 weeks of culture on MS + 0.25 mg/l BA on laboratory benches during the hot summer months when the temperature in the lab was often $30\text{--}32^\circ\text{C}$. Callus had not been noted during earlier maintenance. To determine if the development of callus from shoot cultures is a common characteristic of sugarbeet genotypes, 4–5 shoot culture dishes from each of seven genotypes were cultured at 30°C under dim fluorescent lights ($10\text{--}15 \mu\text{Em}^{-2} \text{ s}^{-1}$). After 70 days, six of the genotypes had developed some callus in the shoot cultures, often as the cultures senesced.

The callus that arose in the 30°C shoot cultures appeared in any of three situations. Some arose on the surface of swollen blackened basal leaves

TABLE I

GROWTH OF SHOOT CULTURE DERIVED CALLUS ON MS MEDIUM WITH AND WITHOUT HORMONES DURING 6 MONTHLY SUBCULTURES

Growth measured as increase in colony area as described in Materials and Methods. Callus was grown at 24°C in the dark.

Genotype	Mean monthly fold increase in growth	Ratio of growth on MS-O ^a to growth on MS-50/50 ^b					
		Months					
		1	2	3	4	5	6
G335-18E	11.4	1.1	1.0	1.0	1.2	1.4	1.6
427-8	14.2	0.9	1.0	1.2	1.0	1.3	1.1
6822-17	16.7	1.0	0.7	1.2	1.3	1.2	0.8
EL 36-18	12.4	1.1	1.0	1.4	1.2	1.9	0.8
EL 36-6	14.3	1.1	0.9	1.5	0.6	1.3	1.1

^aMS medium lacking growth regulators.

^bMS medium containing 0.25 mg/l BA, 50 μ M IAA, and 50 μ M IAA-L-alanine.

of the shoots. This was the predominant mode of origin of the callus of genotype 436-3. A second source of callus was where a leaf touched the agar surface. The tangential portion of the leaf blackened but did not swell. Callus was also found in isolation from any shoot or leaf, although presumably a leaf had been in contact with the agar for a limited time before bending away. In these instances much of the callus growth appears to have occurred after the callus became isolated from the leaf. Irrespective of the site of origin, all callus continued to grow when subcultured from the shoot culture plates. The callus produced rapidly growing (doubling time about 3.5 days) finely dispersed suspension cultures in a variety of liquid media.

Hormone-autonomous nature of callus

Callus initiated in the 30°C shoot cultures was tested for growth regulator dependency by challenging it to continue growth on MS medium without auxin or cytokinin (MS-O) or with a combination of 0.25 mg/l BA, 50 μ M indole-3-acetic acid (IAA), and 50 μ M IAA-L-alanine [14] (MS-50/50). Callus of all five genotypes tested was found to be hormone-autonomous (Table I). Callus of five genotypes grew at approximately the same rates on a medium lacking hormones as on standard sugarbeet callus growth medium (MS-50/50), with no reduction in growth rate during the 6 month test period. This observation was paralleled by measurements of the fresh weight increase of one genotype during three monthly subcultures on MS-O (Table II).

Shoot regeneration from callus

Callus developing in the shoot cultures only rarely displayed leaves or buds. Callus cultured at 20°C under continuous fluorescent light (about 100

TABLE II

FOLD INCREASE IN FRESH WT. OF EL 36-18 CALLUS ON MS MEDIUM LACKING GROWTH REGULATORS, AT 20°C

Monthly subcultures	Fold increase (\pm S.D.)
1st	14.9 \pm 5.2
2nd	20.8 \pm 9.7
3rd	16.5 \pm 8.0

$\mu\text{Em}^{-2} \text{ s}^{-1}$) on MS medium either lacking growth regulators or with 0.25 mg/l BA remained unorganized. When fresh callus from two shoot culture genotypes grown at 30°C under dim fluorescent light ($10\text{--}15 \mu\text{Em}^{-2} \text{ s}^{-1}$) was inoculated onto MS medium with either 0.25, 1.0, or 5.0 mg/l BA in combination with either 0 or 0.3 mg/l IAA and grown under the same light and temperature conditions, the callus of both genotypes regenerated leaf structures and shoots after 28 days at all three BA concentrations, both with and without IAA (Table III). Whole plants were established in soil. Subsequently, callus from shoot cultures of three of four other genotypes was induced to regenerate leaves and shoots on MS + 1.0 mg/l BA + 0.3 mg/l IAA under the same conditions.

Occasionally buds were regenerated on MS-O at 30°C from newly-arisen callus. Additionally, shoot culture callus taken through 3 monthly transfers on MS-O at 20°C regenerated when challenged with MS + 1.0 mg/l BA + 0.3 mg/l IAA at 30°C.

TABLE III

REGENERATION OF LEAVES OR SHOOTS FROM SUGARBEET CALLUS AFTER 28 DAYS ON MS MEDIUM

Genotype	Growth regulators added (mg/l)		Proportion of calli regenerating
	BA	IAA	
EL 36-18	0.25	0	3/8
	1.0	0	7/9
	1.0	0.3	6/9
	5.0	0	4/9
	5.0	0.3	5/9
436-3	0.25	0	1/5
	1.0	0	3/5
	1.0	0.3	3/5
	5.0	0	1/5
	5.0	0.3	0/5

DISCUSSION

The hormone-autonomous sugarbeet callus described in our report differs in several ways from that described by DeGreef and Jacobs [10]. The callus studied in our laboratory is repeatedly obtainable, has been induced from numerous genotypes, and regenerates shoots only occasionally on media lacking cytokinins. The DeGreef-Jacobs habituated line arose in a single culture and is capable of continued shoot regeneration in the absence of growth regulators. Furthermore, the DeGreef-Jacobs line is apparently partially differentiated, whereas our callus is white, friable and appears unorganized. Other work has established that several different cytokinins are effective in inducing this callus from isolated shoot culture petioles and stimulating shoot regeneration from callus [15].

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